

**NON-ENDOGENOUS, CONSTITUTIVELY ACTIVATED
HUMAN SEROTONIN RECEPTORS AND SMALL MOLECULE MODULATORS
THEREOF**

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**GENERATION OF NON-ENDOGENOUS, CONSTITUTIVELY ACTIVATED
HUMAN SEROTONIN RECEPTORS 5-HT2C AND 5-HT2A**

**A. CONSTRUCTION OF CONSTITUTIVELY ACTIVE 5-
HT2C RECEPTOR cDNA**

- 1. ENDOGENOUS HUMAN 5-HT2C**
- 2. AP-1 cDNA**

**B. CONSTRUCTION OF CONSTITUTIVELY ACTIVE 5-
HT2A RECEPTOR cDNA**

- 1. ENDOGENOUS HUMAN 5-HT2A**

2. HUMAN 5-HT2A (C322K; AP-2)
3. AP-3 cDNA
 - (A) REPLACEMENT OF IC3 LOOP
 - (B) REPLACEMENT OF CYTOPLASMIC TAIL
4. AP-4 cDNA

II. EXAMPLE 2

RECEPTOR EXPRESSION

- A. pCMV
- B. TRANSFECTION PROCEDURE

III. EXAMPLE 3

GTP MEMBRANE BINDING SCINTILLATION PROXIMITY ASSAY Y

IV. EXAMPLE 4

SEROTONIN RECEPTOR AGONIST/ANATAGONIST COMPETITIVE BINDING ASSAY

V. example 5

INTRACELLULAR IP3 ACCUMULATION ASSAY

- A. COS-7 AND 293 CELLS
- B. 293 CELLS

VI. EXAMPLE 6

SCREENING OF COMPOUNDS KNOWN TO HAVE 5-HT2C ANTAGONIST ACTIVITY AGAINST NON-ENDOGENOUS, CONSTITUTIVELY ACTIVATED HUMAN SEROTONIN RECEPTORS: AP-1

VII. EXAMPLE 7

SCREENING OF CANDIDATE COMPOUNDS AGAINST NON-ENDOGENOUS, CONSTITUTIVELY ACTIVATED HUMAN SEROTONIN RECEPTORS.

VIII. EXAMPLE 8

SCREENING OF IDENTIFIED COMPOUNDS TO CONFIRM RECEPTOR BINDING: MESULERGINE (ANTAGONIST) COMPETITIVE BINDING ASSAY

FEDERAL REGISTER
Vol. 63, No. 100
Tuesday, May 20, 2008
10000-00000

IX. EXAMPLE 9a

GENERAL SCREENING PARADIGM: SELECTION OF PRE-CLINICAL CANDIDATE LEADS

X. EXAMPLE 9b

CONSTITUTIVELY ACTIVATED HUMAN 5HT2C RECEPTOR (AP-1)

MEDIATED FACILITATION OF GTP γ S BINDING TO COS7 MEMBRANES

XI. EXAMPLE 9c(1)

COMPETITION STUDIES: MUTATED HUMAN 5HT2C RECEPTOR (AP-1)

XII. EXAMPLE 9c(2)

COMPETITION STUDIES: WILD TYPE HUMAN 5HT2A RECEPTOR

XIII. EXAMPLE 9d

RECEPTOR-MEDIATED INOSITOL PHOSPHATE ACCUMULATION

XIV. EXAMPLE 10

RESULTS

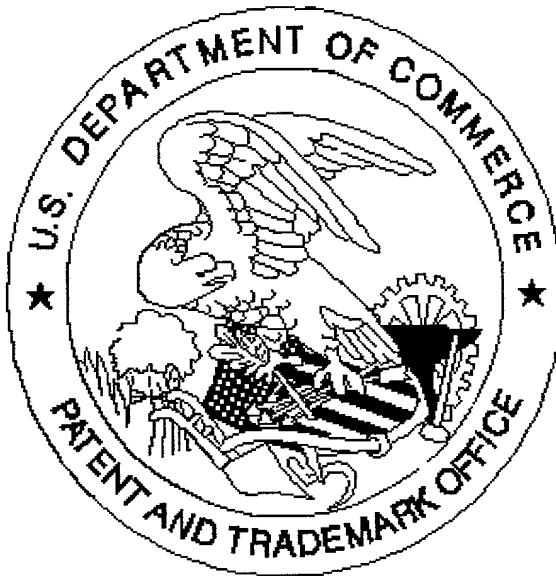
SCREENING CANDIDATE COMPOUNDS

SEQUENCE LISTING

ABSTRACT OF THE DISCLOSURE

FIGURES

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